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RESEARCH ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY OF VIRULENTS AEROMONAS ISOLATED FROM DISEASED FARMED TILAPIA OREOCHROMISNILOTICUS (LINNAEUS, 1758)

Affoue Edwige Kouassi[1], Francis N'guessan[2] and Marina Koussemon[3]

1. UFR-Sciences and Environment Management, Laboratory of Environment and Aquatic Biology,Nangui Abrogoua University,02 BP 801 Abidjan 02, Cote d'Ivoire.
2. UFR-Environment, Laboratory of Biodiversity and Tropical Ecology, Jean Lorougnon Guede University, BP 150 Daloa, Côte d'Ivoire.
3.UFR Food Science and Technology, Laboratory of Microbiology and Biotechnology, Nangui Abrogoua University, 02BP801 Abidjan 02, Côted'Ivoire.

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Abstract

Bacterial isolates were collected from diseased farmed tilapia fish of theMicrobiologyLaboratory,Department of Fisheries and Aquaculture, Nangui Abrogoua University in Côte d'Ivoire. The isolated bacterial strains were identified by using biochemical identification kit API NE (BioMerieux,France).Elevenantimicrobial agentsviz.,chloramphenicol doxycycline, streptomycin, trimethoprim, nitrofurantoin, azithromycin, erythromycin,cefalexin, ampicillin, oxytetracycline and chlortetracyclin were tested by disc diffusion method. The result showed that the majority of the isolated bacteria was Aeromonashydrophila (51,3%). Regarding the sensitivity, all of the Aeromonashydrophila isolates were found sensitive to6 antimicrobial agents(chloramphenicol, doxycycline, streptomycin, trimethoprim, nitrofurantoin, azithromycin). But, 81,8% of the isolates were found moderately sensitive to oxytetracycline. In addition, 100% isolates exhibited resistance to ampicillin and cefalexin. It is notable that there were large numbers of antimicrobial agents sensitive fish pathogenic bacteria in tilapia fish, which showed that the antimicrobial agents represent the weapon of choice against bacterial infections.The use of antimicrobial agentsshould take place under well-defined conditions approved by the relevant public health body.

Introduction:-

Tilapia is a freshwater fish belonging to the cichlid family, originally from Africa, known for its mild flavor, white, flaky flesh and use in aquaculture. It is a popular and inexpensive food source, often farmed and consumed globally for its lean protein content.Tilapia can be prepared in various ways such as grilling, frying and baking. It has an overall production of more than two million tons after carp and salmon (Fitzsimmons, 2006). In Côte d'Ivoire, it is the most consumed fish (Kone, 2015). However, the manifestation of bacterial pathology is a factor limiting the

Corresponding Author:-Affoue Edwige Kouassi
Address:-UFR-Sciences and Environment Management, Laboratory of Environment and Aquatic Biology,Nangui Abrogoua University,02 BP 801 Abidjan 02, Cote dIvoire.

production of tilapia culture.Bacterial pathology is one of the most important diseases in tilapia fish and an important cause of economic loss and fish mortality (Wei etal., 2015). The bacterium Aeromonashydrophila causes high mortality in several farmed fish (Aboyadak, 2015). Among these fish, tilapia Oreochromisniloticus remains one of the preferred hosts of Aeromonashydrophila. This bacterium causes Aeromonas septicaemia sometimes reported in tilapia fish (Roy et al., 2017). Associated clinical signs are the presence of skin haemorrhages, fin erosion and inflammation of the intestine. In addition, according to Boussini et al. (2016), Aeromonashydrophila has been isolated from furunculosis epidemics in tilapia farms in Côte d'Ivoire.Antimicrobial agents are substances that kill or inhibit the growth of microorganisms like bacteria, fungi, viruses, and protozoa. They are classified by their target, such as antibiotics for bacteria or antivirals for viruses and can be synthetic, semi synthetic or naturally derived. Key types include antibiotics like penicillin, antifungals such as ketoconazole, antivirals and antiseptics or sanitizers used on surfaces. Antimicrobial susceptibility testing determines which antimicrobials are effective against a specific bacterium or fungus. Common methods include the disk diffusion test and minimum inhibitory concentration methods, which are used to help healthcare providers choose the most effective drugs for treating infections. In this study, a wide range of antibiotics were analyzed in vitro to determine their potentiel efficacy against bacteria isolated from diseased tilapia fish. The objectives of this study are to study the prevalence of bacterial pathogenic species and to evaluate the antibiotic susceptibility profile of the main bacterial pathogens responsible for the diseases of tilapia fish grown in Côte d'Ivoire.

Materials and Methods:-

Isolation bacteria:

The bacterial isolates used in this study were derived from 76 diseased farmed tilapia fish of the Microbiology Laboratory, Department of Fisheries and Aquaculture, Nangui Abrogoua University. Samples were collected from the fish lesions and homogenized using a pestle and motar for 2 min. The homogenate was enriched in soy trypticase broth (BioRad, France), and incubated at 37 °C for 24 h according to Sakar et al.(2012). After incubation, an aliquot (0,1 mL) of the enrichment was inoculated in Aeromonas agar (Sigma Aldrich, India) containing ampicillin (30 mg/L) and incubated at 37 °C for 24 h. Bacterial isolates were identified using an appropriate type of biochemical identification kits (API NE kits, BioMerieux, France).

Antimicrobial susceptibilitytest:

The antimicrobial susceptibility testfor Aeromonas strains was done using standard antibiotic discs by disc diffusion techniques according to the 2016 edition of the AntibiogrammCommittee of the French Society of Microbiology (CA-SFM). The principle of this test is based on the diffusion of the antibiotics impregnated in the discs within the agar and the determination of the inhibition diameter. DifferentAeromonashydrophila and 11 typesantimicrobial agents were used for the test. The antimicrobial agents tested were chloramphenicol (30 µg), oxytetracycline (30 µg), trimethoprim (25 µg), erythromycin (15 µg), streptomycin (300 µg), chlortetracycline (30 µg), nitrofurantoin (100 µg), ampicillin (10 µg), doxycycline (30 µg), cefalexin (30 µg) and azithromycin (15 µg).

For the preparation of the inoculum, colonies of an 18 h culture obtained on nutrient agar were collected and suspended in10 mL of physiological water (0,85% NaCl) contained in a Mac Farland 0,5 scaleturbidity tube equivalent to approximately 10^8UFC/mL. Mueller Hinton agar was then inoculated with the resulting suspension using the swabbing technique and excessliquid was removed by rotating the swab on the tube walls. After drying the agar plates for 10-15 minutes, the antibiotic discs were applied to the agar surface with forceps and the plates were incubated at 37°C for 24 hours. After the incubation period, the inhibition diameters of the antibiotics appearingaround the colonies were measured using a graduatedruler and the strains were classified as sensitive, intermediate or resistant according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM), (2016).

Results:-

The prevalence of Aeromonas isolates from diseased Oreochromisniloticusare recorded in figure 1. The results show that the majority of the bacteria isolated was Aeromonashydrophila (51,3%). Strains of Aeromonascaviae (18,4%) and Aeromonassobria (30,3%) were also isolated from diseased tilapia fish.Ten isolates of Aeromonashydrophila were screened against 11 antimicrobial agents to determine the prevalence of antimicrobial susceptibility among these isolates (Table 1). All of the isolates showed sensitivity to six antimicrobial agents such as chloramphenicol, doxycycline, streptomycin, trimethoprim, nitrofurantoin and azithromycin.The results indicated 81,8% sensitivity of

Aeromonashydrophila isolates to oxytetracycline. All isolates of Aeromonashydrophila were resistant to ampicillin and cefalexin.

Discussion:-

Aeromonashydrophila was the dominant species identified as the cause of the bacterial disease in the tilapia fish studied. Aeromonashydrophila is the bacterium most commonly associated with aquatic animal diseases (Bullock et al., 2014). These findings may differcompletely or partially with that recorded by many authors and this could be due to abiotic and biotic conditions of the environments where the studies performed.Most of bacterial pathogens in fish farms are normal inhabitant in water and do not develop simply as the result of exposing a host to an infectious agent (Wedekind et al., 2011). The outbreaks of the detected bacterial pathogens were usually associated with change in environmental conditions (stressors), sudden change in temperature, inappropriate handling and physical injury during transportation of fishes.

Environmental stressors can affect the homeostatic mechanism of fish, thus reducing their resistance to pathogenic organisms and triggering the disease outbreaks(Small and Bilodeau, 2005) so inapt farm management systems are prone fish in aquaculture to a variety of diseases(El-Sayed, 2006). Some bacterial fish diseases are uncommon to infect tilapia as Methicillin-resistant Staphylococcusaureusand Aeromonassobriabut due to close contact of aquaculture environment with animal and/or human wastes, these bacteria flourish (Dar et al., 2016).

The isolates of Aeromonashydrophila showed sensitivity to chloramphenicol, doxycycline, streptomycin, trimethoprim, nitrofurantoin and azithromycin. This is interesting because these antimicrobial agents could be used in antibiotic therapy in fish farming. Similar results to those of this study have been reported by other authors. Indeed, Samal et al. (2014) revealed during their work on the antimicrobial agents resistance of Aeromonashydrophila isolated from water that 100% of the strains were sensitive to doxycycline and azithromycin. Similarly, Wamala et al. (2018) reported that 98% of the strains were susceptible to trimethoprim and streptomycin. According to Hafez et al. (2018), resistance to chloramphenicol is extremely rare in strains of Aeromonashydrophila.

Results show 81,8% sensitivity of Aeromonashydrophila isolates to oxytetracycline. These results corroborate those of Elsayed et al. (2024) who reported that isolates of Aeromonas hydrophila were sensitive to oxytetracycline. According to Austin and Austin (2016) and Hamoudaet al. (2019), oxytetracycline is still widely used to control Aeromonas infections. The results of this study are slightly different from those of Revinaet al. (2017) who observed that about 62% and 90% respectively of the isolates were resistant to oxytetracycline. This slight difference could be explained by the fact that isolates can develop resistance to oxytetracycline. This resistance would be related to the inappropriate use of the antimicrobial agents, genetic support, hereditary, and causes serious problems for antibiotic therapy. All isolates of Aeromonas hydrophila were resistant to ampicillin and cefalexin. This shows that no isolates were found to be sensitive to the penicillin and cephalosporin groups of antibiotics. These results could be explained by the fact that the Aeromonas hydrophila germ is naturally refractory to the action of ampicillin and cefalexin.

Table 1 : Percentages of Antimicrobialsusceptibility of Aeromonas hydrophila isolates (n=10)

	Antimicrobial agents sensitivity pattern					
	Sensitive		Intermediate		Resistant	
Antimicrobial agents	N	%	N	%	N	%
Oxytétracycline (30 µg)	9	81,8	0	0	2	18,2
Triméthoprime (25 µg)	11	100	0	0	0	0
Chloramphénicol (30 µg)	11	100	0	0	0	0
Chlortétracycline (30 µg)	10	90,9	0	0	1	9,1
Nitrofurantoïne (300 µg)	11	100	0	0	0	0
Streptomycine (10 µg)	11	100	0	0	0	0
Doxycycline (30 µg)	11	100	0	0	0	0
Azithromycine (15 µg)	11	100	0	0	0	0
Erythromycine (15 µg)	5	45,5	0	0	6	54,5
Ampicilline (10 µg)	0	0	0	0	11	100
Céfalexine (30 µg)	0	0	0	0	11	100

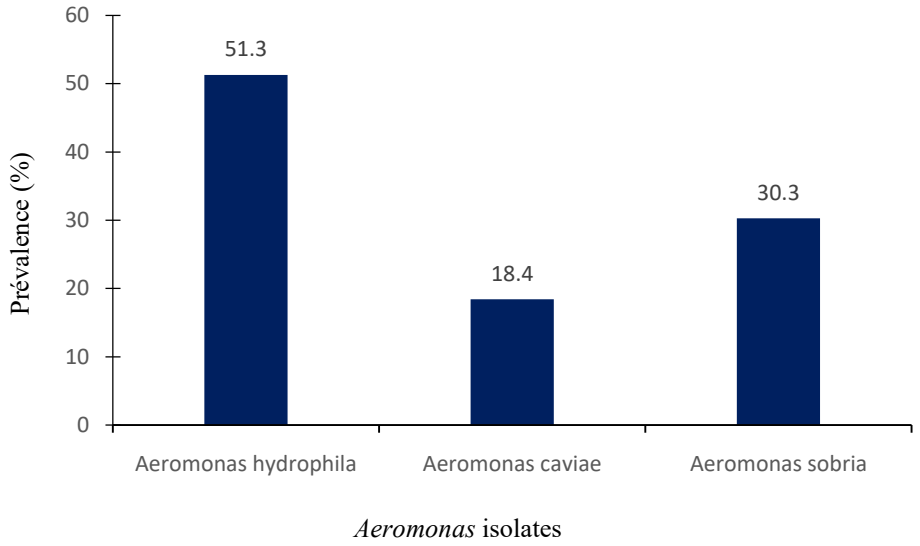

Figure 1 : Prevalence of Aeromonas isolates from diseased Oreochromisniloticus

Conclusion:-

Aeromonas hydrophila bacterium is the most common species of bacteria isolated from diseased farmed tilapia fish. The antimicrobial agents trimethoprim, chloramphenicol, azithromycin, nitrofurantoin, streptomycin, doxycycline and oxytetracycline have a positive effect on the control of Aeromonas hydrophila isolates. These antimicrobial agents could be used as antibiotic therapy in fish farming. In addition, the use of antimicrobial agents requires compliance with simple but strict rules. This is why their use in fish farming is only considered after consultation with a professional to prescribe them, as soon as a doubt is felt or a disease appears.

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